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THE MECHANISM OF CROSSING-OVER

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IT is the object of this paper to give an account of the
most important evidence thus far gained in regard to the
manner in which separation of linked factors—often inter-
preted as "crossing-over"—takes place, and to describe
an experiment in which a new method for studying the
occurrence of such separation is employed. This experi-
ment is still under way, but as it may be a considerable
time before the results are obtained in full, it would not be
advisable to withhold longer an account of this work and
of other work that bears on the nature of the "crossing-
over."

I. THE DISCOVERY OF INTERCHANGE BETWEEN HOMOLOGOUS
CHROMOSOMES

The question as to whether separation of linked factors
is due to pieces of homologous chromosomes changing
places with each other, carries us back to the question
whether the factors lie in the chromosomes at all. As is
well known, there is a large body of evidence from cy-
tology and from experimental embryology, showing that
the chomosomes are persistent, self-perpetuating struc-
tures which have a profound influence upon development.
But the first definite evidence that the Mendelian factors
are contained in the chromosomes lay in the striking cor-
respondence which was found between their respective

methods of distribution—the segregation of Mendelian allelomorphs exactly paralleling the pairing and separation of homologous chromosomes during the maturation of the germ cells, and the random assortment of Mendelian factors belonging to different, independently segregating pairs, paralleling the random assortment of chromosomes belonging to different pairs (Sutton). Still, there was no indication of a connection between any particular chromosome and a particular character until the work of McClung, Stevens and Wilson and others proved that in many animals the "X-chromosome" contains, or at least invariably accompanies, a factor for sex, inasmuch as all fertilized eggs which receive two X-chromosomes develop into females, while those with one X become males.

The next time that particular factors and chromosomes were found to be correlated was in 1910, when Morgan pointed out that the factor in *Drosophila* determining whether an individual shall have red or white eyes, as well as several other factors, must also be located in the X-chromosome (or at least must *accompany* it in its segregation). For, to put the whole argument briefly, the fact that a red-eyed male bred to a white-eyed female produces red-eyed daughters and white-eyed sons shows that the female-producing spermatozoa—those that receive the X-chromosome—also receive the factor for red, but the male-producing sperm—which do not receive the X— also fail to receive red. In other words, the factor for red was judged to be in the X-chromosome, because in the male it is always distributed to precisely the same spermatozoa as those to which the X's happen to be distributed. Bridges has recently obtained evidence that in the female, too, such "sex-linked" factors accompany the X-chromosome in segregation. Ordinarily there is no opportunity for attacking this question in the female, since the female contains two X's, which are of course indistinguishable to the eye, so that it would be impossible to tell whether or not a particular one of the X's was always distributed to the

same eggs as a particular sex-linked factor. But in Bridges's cases of non-disjunction, the maturation divisions are often abnormal, so that some eggs are found to have retained both X's; and in accordance with this it is found that some of the offspring of such females have likewise received two sets of maternal sex-linked factors. These cases, therefore, show that in the female also the sex-linked factors "follow" the X-chromosome (1, 2).

Morgan next studied the relation of different sex-linked factors to each other in inheritance, and then another remarkable fact came to light. Theoretically, the dihybrid females resulting from a cross of a red-eyed fly having rudimentary wings by a white-eyed fly having long wings (both of these pairs of characters are sex-linked) should have contained in one of their two X-chromosomes the factors "red" and "rudimentary," and in the homologous X-chromosome the factors "white" and "long"; the mature eggs should retain either one X or the other and should therefore have contained either red and rudimentary or white and long. In other words, red and rudimentary should be *completely linked* in their inheritance, and similarly white and long. But the results showed that these factors sometimes separate in heredity, for not only the above types of offspring are produced, but also some red longs and white rudimentaries (7); in fact, about 42 per cent. of the offspring belong to one or the other of the two latter classes. If we admit that white and long were originally present in the same chromosome, the *only* way to account for this separation of the factors is to suppose that in some of the cells of the hybrid female the X-chromosomes interchanged parts before being distributed to the eggs. For if the factor for "long" of the chromosome containing "white" and "long" should somehow change places with the "rudimentary" of the homologous chromosome, then when homologous chromosomes are separated at the maturation division, the egg may come to contain either an X-chromosome with white and rudimentary or an X with red and long.

Recent work on *Drosophila* has borne out in a striking way the conclusion that the separation of factors just discussed is due to chromosomal interchange. It will be remembered that the pairs of factors in the example underwent recombination in only about 42 per cent. of the eggs, *i. e.*, they held together more often than they separated, and so might be said to be *partially linked*. Their mode of inheritance therefore forms a contrast not only to complete linkage, but also to the familiar cases of random assortment, where two pairs of factors are found recombined in about 50 per cent. of the offspring, and thus show no linkage at all, presumably because they lie in different pairs of chromosomes which segregate independently. Further investigation showed that not only "white" and "rudimentary," but all the known sex-linked factors, instead of segregating independently, are "partially linked" to one another in greater or less degree. This then was additional evidence that these factors did not lie in different pairs of chromosomes, as in familiar cases, but in the same pair of chromosomes, and that their separation or recombination was therefore dependent upon chromosomal interchange. But furthermore, if these linked factors all lie in the X-chromosome (being sex-linked), then it might be expected that other groups of interlinked factors also would be found, that lie in other chromosomes. A factor in any one of these other groups would not be sex-linked, but would be linked in greater or less degree to every other factor of the same group, since it lay in the same chromosome with it, but it would undergo 50 per cent. of recombination with factors in other groups. This expectation has been fulfilled. In 1911 Morgan and Lynch found two pairs of factors in *Drosophila* (black versus gray body color; vestigial *vs.* long wings) that were linked to each other, but that were not sex-linked (10). These were designated as lying in group II or Chromosome II. Later, Sturtevant found that two other pairs of factors (pink *vs.* red eyes and ebony *vs.* gray body color) were also linked to each other, but were neither sex-linked nor linked to the

other non-sex-linked group; these were assigned to Group III or Chromosome III (15). Incidentally, it was evident that these cases are of exactly the same nature as those previously discovered by Bateson and Punnett in the sweet pea, and termed by them "coupling" or "repulsion." Moreover, the chromosome interpretation made it clear why the factors should be "coupled" or "repelled" according to whether the hybrid received them from the same or from opposite parents. There was only one difference in detail between the facts in the two species: it was discovered by Morgan that in *Drosophila* the linkage is always complete in the male, the separation of factors that are linked to each other occurring only in the female (9); in the plants, on the other hand, recombination occurs in the genesis both of eggs and of sperm.

Since that time the inheritance of over one hundred pairs of factors of *Drosophila* has been studied. This investigation should give an extensive experimental test of the theory of chromosomal interchange, for if linked factors are those carried by the same chromosome, there should be the same number of groups of interlinked factors as there are pairs of chromosomes. There are *four* pairs of chromosomes in *Drosophila*—two pairs of long ones, the pair of moderately long sex-chromosomes, and a pair of very small chromosomes. By 1913, work had been done upon a large number of factors, and the results showed *that all these factors were linked in one of the three groups already discovered*. But in 1914 the author found a pair of factors independent of these (bent *vs.* straight wing), *i. e.*, constituting Group IV (12), and not long afterwards Miss Hoge found another pair of factors in this fourth group (eyeless *vs.* normal eye), (3). Accordingly, the number of groups and of chromosomes now correspond, and not only that, but the relative sizes of the groups correspond in a general way with the relative lengths of the chromosomes. Can it be mere chance that one hundred factors fall into this particular grouping? But if it is admitted that these groups are carried in the

chromosomes, then, as above pointed out, the separation of factors in a group means chromosomal interchange.

II. A Mechanism of Interchange already provided by the Theory of Crossing-Over (Chiasmatype)

Janssen's "chiasmatype theory," based on cytological observations of spermatogenesis in *Batracoseps*, described just such a process of interchange between the homologous chromosomes as Morgan's evidence from genetics required (4). A great bulk of evidence has accumulated to show that during the period of synapsis, homologous chromosomes come into contact, and in many cases chromosomes can be seen to be twisted around each other during one stage or another of synapsis. The essential point postulated by the chiasmatype theory is that, as the paired chromosomes draw apart again, they do not always untwist completely, but may break at some points where they are crossed— thus, in Fig. 1, the upper piece of the light-colored chro-
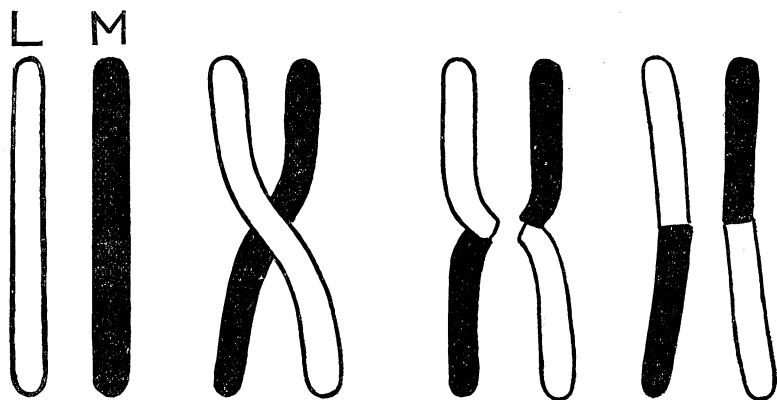

Fig. 1.

mosome (L), which was on one side, loses its connection with the lower part of L, that has crossed to the other side, but becomes united instead with the lower piece of the dark chromosome (M) which, on account of the crossing of the strands, now follows it on the same side; similarly, of course, the upper part of M becomes united with the

lower part of L; in this way a recombination of parts is accomplished.   Morgan and other workers on *Drosophila* base their acceptance of this essential point in Janssens's chiasmatype theory upon the evidence (from cytology) that homologous chromosomes do twist about each other during synapsis, taken together with the evidence (from genetics) that these chromosomes emerge as new combinations.   Janssens, on the other hand, maintains that certain details in the appearance of the chromosomes during that stage in synapsis called ''strepsinema'' give ocular evidence that crossing-over occurs at this particular period and in a particular manner.   As it would seem possible, however, to put another interpretation upon his figures, this question may be deferred until later.

Janssens had intended the chiasmatype theory to explain the supposed fact that there might be more pairs of factors
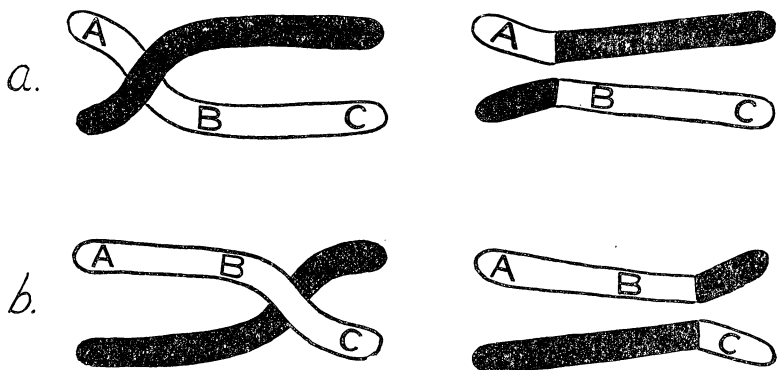
FIG. 2.

capable of recombination than there were pairs of chromosomes.   (It might be mentioned in passing, however, that at that time this fact had not yet been demonstrated; there are even now probably no published facts except those recently discovered in *Drosophila* which prove this point).   As shown above, Morgan went further than this with the chiasmatype theory by applying it to explain, specifically, the recombination of *linked* factors (8).   Moreover, he pointed out at the same time an important corol-

lary to this theory. It has already been stated that he had
found different degrees of linkage to exist between the
various factors of a group: for example, the proportion
of cases in which separation occurs between white and
rudimentary was said to be 42 per cent., whereas the fre-
quency of separation between white (eye color) and the
factor for yellow body color is only about 1 per cent. In
explanation of these different degrees of linkage, Morgan
pointed out that, on the chiasmatype theory, the closer the
proximity of two factors to each other in the chromosome,
the smaller would be their frequency of separation, for
the less would be the chance for a crossing-over of the
chromosomes to occur *between* them. Thus, in Fig. 2, the
factors A and C become separated both in case (*a*) and
in case (*b*), because A and C lie so far apart that in both
cases the point of crossing-over falls between them, but in
only one of the cases do A and B separate, and in one case
B and C, since these are so near together that the point of
crossing-over may often be beyond instead of between
them. In other words, on the chiasmatype theory, fre-
quency of recombination must be, to a certain extent at
least, an index of the distance apart of factors along the
chromosome. Since the time when these ideas were pro-
posed (1911), two important series of facts have come to
light in the studies on *Drosophila,* in support of the chias-
matype theory of interchange and of these extensions of it.

III. A Verification of the Theory of Crossing-Over. The Law of Linear Linkage

It occurred to Sturtevant in 1911 that, if the factors
are carried in the chromosomes, then, *owing to their linear
arrangement*, the distance along the chromosome between
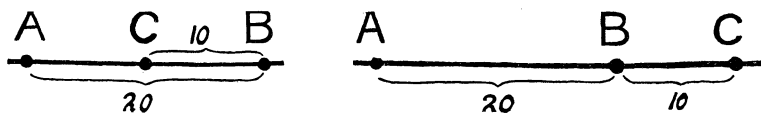any two factors (A and C) must be either the sum or the

Fig. 3.

difference of their distances from any third factor (B) of
the same group, *i. e.,* length AC = length AB ± length BC,
the + or — depending on whether the third factor is be-
tween or beyond the other two (see Fig. 3). Accordingly,
if, as Morgan suggested, the frequencies of separation
(linkage values) between factors depend on their distances
apart, then the frequency of separation (degree of link-
age) between the two factors, A and C, should be predict-
able, given the frequency of separation of each from the
third factor, B. To put the matter diagrammatically, A,
B and C have been represented in figure 3 as points along
a line; A and B, we will suppose, separate from each other
in heredity in 20 per cent. of cases, to correspond with
which they have been placed the same number of units (20)
apart in the diagram; similarly, B and C, which we will
suppose to separate 10 per cent. of the time, have been
placed 10 units apart. (As above pointed out, there are
obviously two possible diagrams to choose between, de-
pending on whether C is beyond A and B or between
them.) Then, if it be true that the frequency of separa-
tion between any factors is always precisely proportional
to their distance apart, it will follow that the per cent. of
separations between A and C will be equal to the number
of units of distance on our diagrammatic chromosome be-
tween A and C; this in turn equals AB ± BC = 30 or 10.
If separation frequency bears a less simple relation to dis-
tance, but is nevertheless determined by it (see below),
frequency AC will not *equal* distance AC (*i. e.,* AB ± BC)
but can be *calculated* from the latter. On the other hand,
if our premises are false, and there is no linear relation at
all between the factors that determine their frequency of
separation, then frequency AC will not be equal to dia-
gram distance AC (*i. e.,* to AB ± BC), nor even, in the
case of different sets of factors, will it bear any constant
relation to diagram distance AC; that is, it would not be
possible to discover any constant rules for calculating the
third frequency from the two others which will hold, even
approximately, for various sets of factors (BCD, LMN,
etc.).

Sturtevant found that there is indeed a linear relation in the frequencies of separation (14, 16). In the case of smaller per cents. of separation, per cent. AC always is precisely equal to the sum or difference of per cents. AB and BC (within the limits of probable error), so that the per cents. of separation for all combinations of these factors is accurately represented by a linear diagram. In the case of higher per cents. of separation (long distances), the highest of the three frequencies (let us call it AC) falls short of the sum of the other two (AB + BC), and so it is a smaller number than the distance representing it on the diagram, but it nevertheless (within the normal limits of error) can be calculated from this diagram distance AC, for a constant relation was discoverable between this hypothetical distance and the actual frequency. Thus the different frequencies do not bear any random relation to each other that is mathematically possible, but bear relations that disclose a linear connection between the factors.

It remains to consider the meaning of the fact that in cases where there is a high per cent. of separations, the highest per cent.—that between A and C, let us say—is not as great as the value of the distance AC representing it on the diagram, *i. e.*, it is less than the sum of the per
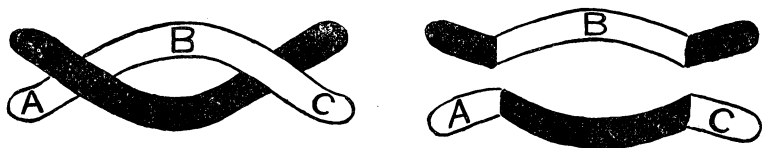
Fig. 4.

cents shown between A and B, and B and C, respectively. If, whenever A and B or B and C separate, A and C separate also, as shown in Fig. 2, (*a*) and (*b*), then per cent. AC would be equal to per cent. AB plus per cent. BC, but since per cent. AC is lower than this, there must be cases in which, although A and B or B and C separate, A and C fail to separate. It is obvious that in these cases, where B separates from A but A does not separate from C, that B

must have separated from C also, *i. e.,* a separation has occurred between A and B, and between B and C, coincidentally. On the view above presented, a separation means a crossing-over of chromosomes, and so in these cases the chromosomes must be thought of as crossing-over at two points coincidentally, as shown in Fig. 4. This process has been named by Sturtevant "double crossing-over." As shown in the figure, where crossing-over occurs coincidentally, both in AB and in BC, the chromosome crosses and crosses back again between A and C, hence the latter factors do not become separated.

When the frequencies of separation (diagram distances) between A and B, and between B and C are both small, it is to be expected as a matter of pure chance, if the factors are joined in line in the manner described, that such coincidences will occur very rarely, even in proportion to the small frequencies involved, and so the per cent. of separations between A and C will be practically as great as that between A and B plus that between B and C. Hence per cent. AC will be accurately represented by diagram distance AC. On the other hand, if separation is frequent between A and B or between B and C, there should be more chance of coincidence of these separations, and the number of separations between A and C will fall correspondingly short of AB + BC, which is the value of AC shown on the diagram. Consequently, in predicting frequency AC on the basis of AB and BC, allowance must be made for these coincidences. But the author ventures to point out that, as the number of these coincident separations is found to be largely determined by the frequency of separation, greater frequencies being accompanied by a larger proportion of coincidences, as has been shown, then the amount of allowance to be made can be approximately calculated for any given distance on the diagram; accordingly, the frequency of separation between A and C can be calculated from AB + BC, *i. e.,* from the distance AC on the diagram. The precise manner in which coincidence of separations increases with their frequency is a

question which will be reserved until later. But it is clear that, since coincidence does not vary independently of ''distance,'' a linear relation holds between the linkage values, in that these values can be calculated from a linear diagram much more exactly than would be expected on chance relationships; in mathematical terms, the frequencies of separation between all combinations of the different factors in a group are largely a *function* of the distance apart of these factors in a linear figure.

It will now be desirable to consider these same facts from another angle. As it is possible to represent the linkages between *any three* factors of a group in terms of their distances on a linear diagram, it follows that *all* the factors of a group together can be represented in one linear diagram. Suppose that such a diagram has been made,,and that the order of the factors in it is ABCDEFG. Now, as has just been explained, since per cent. AC nearly equals per cents. AB plus BC, it must follow that a separation between A and B rarely coincides with one between B and C; the same fact may also be expressed by saying that when A and B separate, C stays with B rather than with A. Similar relations, of course, hold for the other factors, too; thus D also stays with B and C when A and B separate, but it stays with C when B and C separate. The linkage of D with B, then, is only due to its linkage with C, for, although it usually stays with B, it very rarely stays with it except when C does. Thus D is linked to B only through C, and to A only through B. Similarly, all the other factors also are linked together in a chain, each to the one on either side: just as D is linked on the one hand to E, and on the other hand to C, but not to any other factors except through one of these, so C is linked on the one hand to D, on the other hand to B, but is linked to E only through D, and to A only through B, etc.; moreover, all the factors are linked to the others in the same order. Separation of factors in such a group accordingly means the breaking of the chain at just one or two points, for it has been pointed out that when B and C separate, A and

B rarely separate coincidentally, but usually remain to-
gether, and C, D, E, etc., all remain together also, separa-
ting in a body from A and B. In other words, the factors
are not interchanged singly, but stay together in sections,
according to their positions on the diagram, and whole
sections are exchanged at once.

It may be objected that these conclusions are in many
cases based on linkage values obtained in different experi-
ments; that it is unwarranted to conclude, for instance,
that when C and D separate, E remains with D, simply
from the fact that in one experiment the frequency of
separation between C and D had the value m per cent., in
a second experiment DE was n, and in a third experiment
CE was m + n, for this conclusion would only be true on
the supposition that in all three experiments each factor
had the same frequency of separation with each of the
others as in the particular experiment where that fre-
quency was determined. The answer is that numerous
experiments have been performed in which three pairs of
factors (or more) could be followed at the same time, and
these experiments have given results precisely the same
in kind, although more accurate than the preceding. But
in experiments of the latter type, the coincidence of the
various separations and non-separations does not have to
be calculated out as in the case above, but is given directly
by the results. Thus in a hybrid which has received ABC
from one parent and the allelomorphs abc from the other,
gametes in which coincident separation between B and A
and between B and C has occurred will be distinguishable
by having either the composition aBc or the composition
AbC (see Fig. 3), and the number of such offspring can
thus be directly counted instead of it being necessary to
calculate them from the relations between separation
values for A and B, B and C, and A and C. And in the
experiment of the author's given in Section V, where the
inheritance of a large number of factors is followed simul-
taneously, the results show directly and graphically that

the factors, as arranged in line in order of their linkage, are exchanged in whole sections at a time.

In the first section, evidence was presented, showing that groups of factors are connected with particular chromosomes, and segregate with them at the maturation divisions; this was in fact *proved* to be true in the case of sex-linked factors, which are found always to segregate with the X-chromosome during spermatocyte divisions. Yet it was conceivable that the factors were not actually *in* the chromosomes, but rather tied to them by some obscure connection (chemical, physical or metaphysical), although the fact that the relative sizes of the groups correspond to the lengths of the chromosomes might be taken as evidence against such a view. On that view, a separation of linked factors would be considered not a physical interchange between the chromosomes themselves, but a transference, by a factor, of its invisible bond, from one chromosome to the homologous one. But Sturtevant's evidence just presented shows that however one may have conceived, *a priori,* the chemical attraction or physical connection that makes linked factors tend to segregate to the same pole in the maturation divisions—this connection binds them in a *linear manner,* one after another, in a chain. This unique result, then, constitutes specific evidence that the factors are actually in the chromosomes, in an order which can be determined by their linkage relations, and that the separation of linked factors is consequently a real interchange between parts of the chromosomes themselves.[1]

[1] The fact of linear linkage does not connote that frequency of crossing-over is necessarily entirely dependent upon distance, for it is still possible to escape the conclusion that crossing-over occurs equally often in all parts of the chromosome, by assuming that coincidence of separations A–B and B–C usually occurs with not very different frequency from coincidence of separations G–H and H–I, even if different actual lengths are involved, provided the frequencies of separation are the same in the two cases. And on either way of explaining the results, the factors must be linked in line in the chromosomes in the same order as on the diagram. In fact, no matter how great the differences in frequency of crossing-over in different parts of the chromosome might be, the linkage order of the factors would still be the same as their real order so long as coincident crossing-over in any two regions did not occur as often as single crossing-over in either region.

Furthermore, admitting this conclusion that the linkage diagrams really represent the chromosomes, the fact that the factors are exchanged in sections proves that whole pieces of the chromosomes change places at once, as occurs in the process of ''crossing-over'' postulated by Jannsens, instead of small parts or factors in the chromosomes being separately exchanged. The idea that the interchange during synapsis may be a kind of exchange of separate particles from one container to another seems to have been held by a number of geneticists. On this view, the chromosomes might be considered as a sort of pod, containing the factors within them like so many beans; when the chromosomes synapse, the pods open towards each other, so that a factor in one might change places with a factor in the other. Conceivably—if we adopt this view—certain factors might be harder to dislodge than others, and so different frequencies of separation would exist between different factors. But such a mechanism of interchange would not result in a mode of linkage that may, in the sense explained above, be called linear, for separation of factor B from A would, on this mechanism, have no influence at all on whether or not C separated from A. This difficulty could be partially met by supposing that interchange of one factor in some way facilitates interchange of the neighboring factors, but the type of linkage which is actually found goes much further than this, and shows that the whole group of factors remains intact except at one or two points, interchange being in two or three entire sections. This can only mean, then, that interchange is a process of *crossing-over,* if it occurs by means of synapsis at all.

It might be claimed, however, that this interchange of whole sections of the chromosomes need not occur at synapsis, and therefore need not be of the nature of crossing-over at all. The only alternative to crossing-over, however, would be to suppose that, during the resting period of cells, the chromosomes might break up into pieces, and that then, in reuniting, a fragment of one chromosome

might become joined with a piece of the homologous chromosome instead of with a piece of the same chromosome. But on the fragmentation theory it must be supposed that the fragments reunite in exactly the original order, and, further, that the two homologous chromosomes break at precisely the same point before interchanging—otherwise one reformed chromosome would lack certain factors and the other would have too many; nevertheless, this point can not be a fixed point, as interchange may occur anywhere. Since interchange, when it occurs, usually takes place at one point only, it must also be assumed that the frequency of the recombination just described is so nicely regulated that in about half of the cases it has happened just once (and at one point in the chromosome) during the sum total of resting periods of all cells ancestral to any particular egg cell that shows interchange. For in about half the eggs a particular chromosome has exchanged in only two sections, and in very few have there been more than three points of interchange. Moreover, in the ancestry of the rest of the eggs, no interchange whatever can have occurred. Finally, the fallacy of the fragmentation idea becomes obvious when we consider that if interchange took place in the resting period of an embryonic cell, most of the eggs derived from this cell would show that particular recombination, and hence the individual in question would give an unusually large proportion of offspring of this sort. Thus different individuals of the same strain would differ greatly in their linkage values, there being scarcely any constancy at all. Since this is not true it would have to be assumed that interchange takes place only a short time before the maturation divisions, owing to some peculiarity in the chromosome processes occurring in the cells at this period. Thus we return again to the conclusion that interchange occurs during synapsis.

Further evidence that interchange occurs during synapsis is to be found in some results obtained with Bridges' ''non-disjunctional'' flies. Non-disjunctional females of

Drosophila contain, besides their two X-chromosomes, a Y-chromosome (owing to previous mitotic abnormalities). The presence of the extra homologous chromosome in some way causes the X's, in some of the oocytes, not to separate properly at the reduction division (presumably, this is because they did not pair with each other as usual, but one of them paired instead with the Y, leaving the other X free to go either to the opposite or to the same pole as the first X). Thus some of the eggs in which the above process has occurred come to contain two X-chromosomes, whereas normal eggs contain only one. Now, it is found that in those eggs which receive both X's, no interchange has taken place between them, whereas in the eggs containing one X, interchange has taken place about as often as usual. Hence interchange is connected with whether or not the Y allows the two X's to unite and separate properly, *i. e.*, interchange seems to be a result of the way in which chromosomes pair and separate during synapsis, and, as we have seen, if interchange occurs at this period, it must be by crossing-over.

B. The Correspondence between Separation Frequencies and Chromosome Lengths

In the present section still another possible test will be given of the conclusions arrived at by Morgan, that the factors are in line in the chromosomes, and that the order in which they lie determines in a general way the relative frequencies with which they separate from one another. And it has just been explained that evidence for these ideas is also evidence for crossing-over: that if the diagrams do represent the chromosomes and show the factors in their real order, then the facts of linkage demonstrate that, during synapsis, whole sections of the chromosomes change places at once, *i. e.*, cross-over.

The second test of the validity of the chromosome diagrams is as follows: If the order of the factors shown by their linkage relations, and represented in the diagrams, is their real order in the chromosomes, then it would be

possible, by adding together the frequencies of separation
between all *adjoining* factors, to obtain the *total* frequency
of crossing-over in the chromosome. This total frequency
would be represented in the diagrams by the total length
of the latter, since it is always the per cent. of separations
between the most closely adjoining factors which is chosen
to determine the number of units of length in any region
of the diagram. Now, the total frequency of crossing-
over in a chromosome ought, we should expect, to be de-
termined by the length of that chromosome. Accordingly,
we should expect to find differences between the total fre-
quencies of interchange (or the diagram lengths) of the
different groups of factors exactly paralleling the size
differences existing between the chromosomes themselves.
It will be seen, however, that such an expectation assumes
also (1) that crossing-over occurs with equal frequency
in all parts of a chromosome, and in equal parts of dif-
ferent chromosomes, and (2) that the factors available
for working out the total frequency of interchange do not
lie in any one limited region of the chromosome, but are
more or less scattered, some of them lying near each end.
A negative result from our test, then, might merely mean
that one of these two assumptions was incorrect, and this
would not disprove any essential point in the theory of
crossing-over previously outlined. On the other hand, a
positive result would seem to be too much of a coincidence
to happen by mere chance, and so would seem to prove the
correctness both of our main theory and of the two latter
points.

In regard to the size relations existing among the chro-
mosomes themselves, as determined by cytological ob-
servations, the work of Stevens (13), taken in connection
with the later work of Metz (5), and of Bridges (2), shows
that there are four pairs of chromosomes in *Drosophila:*
a pair of moderately long sex chromosomes, two pairs of
very long "autosomes," and one pair of minute "auto-
somes."

We may next consider the lengths of the genetic groups,

as determined by their "total frequencies of interchange." The length of the first, or sex-linked, group of factors has been found to be about 66 units; a unit, it will be recalled, is a section of the chromosome of such length that breakage occurs within it, on the average, one time in a hundred cases. The evidence then shows that, in a hundred cases, the first group breaks 66 *times*. This does not mean that it breaks in as many as 66 *cases* out of 100, for it may break two, or, very rarely, even three times, coincidently (at different points along the chromosome) in the same case ("double or triple crossing-over"). As previously explained, when two breaks thus occur coincidentally, the extremes of the chromosome come to lie on the same side, and so a factor at one end of the first group does not separate nearly 66 times in a hundred from a factor at the other end; owing to these coincident breaks it really separates in only about 45 per cent. of cases. The number 66 is consequently not obtained by merely determining the frequency of separation from each other of the two most frequently separating factors, but, as mentioned above, it must be derived by adding together the frequencies of all the smallest parts of the chain (frequencies of $AB + BC + CD$, etc.). In the case of the first group, the determination of this "total-length" has been accomplished by the combined efforts of a large number of people, although by the work of Morgan, Sturtevant and Bridges particularly.

Group II has a much greater length. It is probably over a hundred units long, and is certainly over 90. This result has been obtained principally by the work of Bridges and Sturtevant, although, as before, others have helped very materially. Mention must here be made of the fact that Sturtevant has discovered in this group specific mutant factors which, when *heterozygous,* lower the frequency of separation in certain regions of the group very much, although the order in which the factors are linked is not changed (16). The variation certainly proves, however, that (if the groups represent the chromosomes)

then, under certain special conditions of heterozygosis, different regions of the same chromosome may differ in regard to the frequency of crossing-over within them, for different regions were not affected in the same way by these factors; it also shows that equal lengths of different chromosomes may have different frequencies of crossing-over, for these factors affected only group II appreciably.

In group III, crosses involving several combinations of different factors have been made by Sturtevant, Bridges and Dexter, but the order of none of the factors has until recently been worked out by them nor has any consistent general scheme been attempted. The information has, in fact, not been adequate for this purpose, and much confusion has also arisen on account of the great linkage variation in this group, which seemed to occur very frequently. Sturtevant, who, as stated in section I, discovered the first case of linkage in the third group—namely, that between pink and ebony—had determined the initial positions of these factors, placing them at about 4 units apart, and next Bridges, who had found kidney (eye), had determined its position at about 15 units from pink, though he did not determine the relation between kidney and ebony. As a matter of fact, however, the kidney determination had been made with flies in which there was a greater frequency of crossing-over than in the experiments of Sturtevant, and, as will appear later, in any given experiment kidney is really nearer to pink than is ebony. From time to time after this other mutants were discovered (peach, Bridges, May, 1913; sepia, Wallace, May, 1913; spineless, Bridges, May, 1913; deformed eye, E. Cattell, 1913; band, Morgan, 1913; rough, Muller, June, 1913; sooty, Sturtevant, Oct. 1913; spread, Dexter, Nov. 1913; dichæte, Bridges, July, 1915), and the fact that these mutants were members of the third chromosome group was determined (peach, sepia, spineless, band and dichæte by Bridges; deformed by Cattell; rough by Muller; and sooty by Sturtevant). The author mean-

while undertook experiments with a view to determining
the order of these factors, their frequencies of separa-
tion, and the manner in which these frequencies vary, and
also sought to correlate with the results the data previ-
ously obtained.

It has developed from this work that group III is of
the same "order of magnitude" as the first and second.
This is the result required by the cytological facts. To
complete the parallel, it should be found that the third
group is longer than the first and, in fact, of just about
the same length as the second group. Whether this is
true can not yet be stated definitely, but the results indi-
cate that it is. It is certain that the length of the group
of factors dealt with is at least 55, but another estimate,
which, for reasons given below, would seem more prob-
able, gives the length as over 100. It should also be
borne in mind that not as many factors have as yet been
worked with in this group as in the other two, and it may
well be that other factors will be found to lie beyond any
of the twelve which have so far been approximately
"placed." Thus, even if 55 should be the normal value
for the factors *dealt with*, the whole group may very well
be considerably longer. In the first and second groups,
factors lying well beyond all the others were discovered
after the positions of more than a dozen had already been
determined.

The reason for the uncertainty in regard to the total fre-
quency of separation among those factors which have
been worked with is to be found in the linkage variation.
Sturtevant had discovered that certain races, containing
the mutant factor for ebony body color, gave extremely
low frequencies of separation; that is, ebony flies, when
crossed to those with pink eyes (pink is also in group
III), gave a hybrid in whose germ cells very little recom-
bination between pink and ebony occurred. Other races
(*e. g.*, those containing sooty, an allelomorph of ebony),
when crossed to pink, gave higher values. He therefore
concluded that the ebony flies contain a factor (let us call

it C) which reduces the frequency of separation, and which is dominant, since it produces an effect in the hybrid. I have found that two other races of flies, one having the factor for spread wings (also in group III), and the other showing no "visible" mutant factors, also contain C, as they behave in the same way as ebony. However, the hybrids produced when these races are crossed with ebony give high frequencies of recombination again! This result shows that, as in group II, these races do not really contain a factor which normally reduces separation frequency, for, when both homologous groups of an individual contain the factor C—*i. e.*, when it is homozygous— the frequency of separation is high again. (This also explains an irregularity observed by Dexter, who obtained a high frequency of separation in a cross involving ebony flies.)

It happens, however, that these high separation frequencies obtained when C is homozygous are even higher than those occurring in crosses not involving C at all, and so presumably homozygous for its allelomorph, c. By analogy with Sturtevant's findings in the second group, this would mean that in most crosses hitherto made not involving C there has nevertheless been another factor heterozygous, which has a similar, but lesser, effect on the regions of the group studied. Some support for this interpretation is found in the fact that occasionally higher frequencies are obtained in these crosses not involving C, which appear to overstep the limits of chance variation. The evidence thus far secured on groups II and III points to the conclusion that the highest frequency obtained is that which should be regarded as the normal value, and that very marked departures from this, which affect only a particular group, are generally due to heterozygosis in special factors of that group. If it should be found that marked differences affecting the total frequencies of particular groups do occur, in cases where the flies are homozygous for whatever factors influencing linkage they may contain, we might naturally expect that

such variations would have gradually accumulated in the course of evolution, until no correspondence remained between the relative lengths of the chromosomes and the total separation frequencies.   But the parallel which does exist between the observed chromosome lengths and the usual (homozygous) total frequencies, would seem too close to be meaningless, and so we should be led to believe that for some reason marked variations in the frequencies of particular groups, even though they may be possible, do not generally persist; in other words, the frequencies seem usually to stay at least roughly proportional to the actual chromosome lengths, and to furnish another verification of the theory of crossing-over.   Further evidence of this will be met with when we consider group IV.

As the data whereby the positions of the factors and the total frequency of separation have been determined in group III, have not hitherto been published, it may be of interest to present some of them here.   In order to obtain data on as many combinations of factors as possible in the same cross, so that the linkage values between the different factors would be comparable, I have endeavored to make up, by cross-breeding, stocks containing six or seven mutant factors in group III at the same time.

Since on account of the baffling linkage variation, the order of these factors could not well be determined by combining the results of separate experiments each of which dealt with only two factors at a time, it required a great many trial matings before such multiple stocks could be made up, as of course the crosses have to be made in a certain precise order, to secure a combination of many linked factors.   To obtain stock ABC, for example, it would not suffice to make up AC and then mate it to B, for it would then require two coincident recombinations (which might never occur) to secure ABC.   Moreover, as a first step it had been decided to get combinations of ebony with various factors, and very much time was lost in this attempt, as it was not then known that

when ebony is crossed to most other stocks recombination of the factors is nearly impossible. A stock has finally been obtained, however, combining the following characters belonging to group III: sepia eye color, dichæte (bristles, and wing), pink eye color, spineless body, kidney eye, sooty body color, and rough eye. Data have not yet been secured with this final stock, but the following experiment, in which most of these factors were involved, may be regarded as typical of crosses not involving factor "C," and consequently giving moderately high frequencies of separation. Hybrid females from a cross of sepia flies with flies containing dichæte, spineless, kidney, sooty, and rough, were backcrossed to the quintuple recessive stock—sepia, spineless, kidney, sooty, rough (dichæte being a dominant). The count of offspring is shown below. The classification as regards kidney has not been given, as this character can not be distinguished with certainty in eyes which are also rough.

No Separation

se	dic sps so r
131	109

Separation Occurring at a Single Point

1. Between positions of se and dic		2. Between dic and sps		3. Between sps and so		4. Between so and r	
se dic sps so r	normal	se sps so r	dic	se so r	sps dic	se r	sps dic so
9	16	20	15	23	19	40	28

Separations Occurring Coincidently at Two Points

1; 2. Between se and dic; dic and sps		1; 3. Between se and dic; sps and so		1; 4. Between se and dic; so and r	
se dic	sps so r	se dic sps	so r	se dic sps so	r
1	2	1	2	3	5

2; 3. Between dic and sps; sps and so		2; 4. Between dic and sps; so and r		3; 4. Between sps and so; so and r	
se sps	dic so r	se sps so	dic r	se so	dic sps r
0	0	2	3	1	1

In addition, 1 sooty spineless fly appeared, which must have resulted from a separation at three points coincidently ("triple crossing-over"), namely, between dic and sps, sps and so, so and r.

The above classification of the flies, in respect to where separation of factors occurred, is based on the assumption that the factors are linked in the order: se-dic-sps-so-r, as on any other arrangement the above results would show many more coincident separations between certain factors, than single separations. The reader may convince himself of this by working out the numbers of the different kinds of separations on any other scheme. We may say, then, that the above results prove that the factors are linked in the order just given. Turning to the individual separation frequencies, it will be seen that se and dic separated 25 times when there was no other point of separation, and 14 times when there was a coincident separation, *i. e.,* 39 times in all. As there was a total of 432 flies this means that these factors separated in 9 per cent. of cases, *i. e.,* are 9 units apart. In a similar way the results show that the distance between dic and sps is 10, between sps and so is 11, and between so and r 19.5, giving a total distance between se and r of $9 + 10 + 11 + 19.5 = 49.5$, which agrees well with the value 55, obtained by combining all the records of crosses of this general sort.

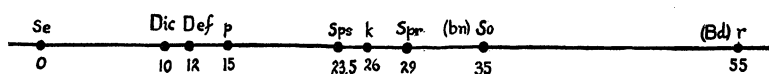| Factors Involved	"Distance" Between Them	Number of Flies on which Result is Based
Sepia dichæte	9.7	624
Dichæte spineless	11.0	683
Sepia spineless	23.4	1,014
Spineless sooty	11.7	1,198
Sooty rough	19.6	1,097
Pink spineless	8.5	825
Pink kidney	11.1	963
Kidney sooty	9.6	885
Pink sooty	19.9	1,566
Deformed pink	3.0	166
Kidney band	8.4	237

FIG. 5.

The following summary of "distances" between various factors of the third group (considered two at a time)

was obtained by averaging together the results of various experiments not involving C, in which I had followed the inheritance of both of the factors listed at the same time. Many of the data listed in separate lines are of course derived from the same experiment, as in most experiments more than two pairs of factors were followed. It should also be added that in one of the cases below (se sps), the distance was slightly lengthened to allow for coincident separations, the approximate proportion of which was known, but the actual number of which had not been determined in most of the crosses.

Having determined the order of the factors, these results may now be combined, in order to obtain a series of values based upon as many data as possible, and to construct a diagram of the group. The diagram so made is shown in Fig. 5. The numbers underneath the symbols of the factors represent the ''distance'' of the latter from sepia, which, as it lies at one end of the group, is used as a common point of reference. Although the distances shown will undoubtedly be subject to revision, the order of all the factors shown, except deformed, band, and beaded, is certain. Deformed (eye) is surely between sepia and pink, but it is not yet quite certain that it is to the right of dichæte; band (thorax) is near sooty, but on which side is not known; beaded (wing) is very near rough, but it has not been established whether it comes before or after it (a count of 50 flies showed no crossing-over between them). It was Sturtevant who first determined the position of beaded (found by Morgan, May '10) with reference to this series (to the right of sooty), and Bridges who first determined that dichæte lies between sepia and pink (about 4 to the left of pink). The data listed merely confirm these findings, so far as these two factors are concerned. And it may here be repeated that numerous other crosses of factors in this group have also been made by these investigators, although the crosses have not been of a sort to show the arrangement of the factors studied.

We may next consider the disposition of the factors of group III in a diagram based upon data from flies heterozygous for C. The separation frequencies which I have obtained are given below:

Factors	Per Cent. of Separations	Number of Flies
Sepia spineless	20.9	527
Spineless kidney	0.0	527
Pink kidney	1.5	868
Kidney sooty (or ebony)	0.2	674
Sooty (or ebony) rough	0.0	843
Kidney rough	0.1	1,211

A diagram based upon these data would show sepia at 0, pink at 19.5, spineless and kidney at 20.9, sooty and rough at 30, and the total length would thus be 30. The tenth of a unit of distance between kidney and sooty is based upon one fly, in which separation had taken place between these factors. Tests of the fly (which was a sooty rough, resulting from a backcross of a female containing p sps k so r from one parent and spread C from the other) showed that the factor C had remained with spread, and that no recombination had taken place between the positions of spread and sooty. This one fly, therefore, proved that both spread and C were to the right of kidney. The factor C is thus seen to lie right in the heart of the region where it exerts its maximum effect, as Sturtevant has also found in the case of the similar factors in group II.

Sturtevant has obtained slightly higher frequencies of separation between pink and ebony in some of his crosses heterozygous for C. The lower value here recorded may then be due to the flies being heterozygous for another factor besides C which disturbs separation frequency, and which is also met with in the crosses not involving C.

The following values have been obtained in crosses in which the factor C was homozygous:

Factors	Per Cent. of Separations	Number of Flies
Sepia pink	27.2	136
Pink ebony	48.0	290

Besides this, it is found that spread is about two thirds of the way between pink and ebony. Ebony, it will be remembered, is an allelomorph of sooty, and therefore occupies the same position. As no recombination has yet occurred between C and rough in flies heterozygous for C, it has not been possible to obtain these factors together and so, in crosses homozygous for C, the linkage of rough has not yet been discovered. The length of the group between sepia and ebony is 75 in these flies, as will be seen from the above data. Although these figures are based on a relatively small number of flies, the difference between this and the shorter value (35) found in flies not containing C is marked enough to be significant, especially since it occurred in various crosses of this sort. If the distance between sooty and rough is expanded in the same way, the group would have a length of much over 100. If, however, this distance is of the same length as in flies without C, the total length would be 95. The reasons have been given which incline us to the opinion that these values obtained in crosses homozygous for C may represent the "normal" figures for this group rather than those obtained in the experiments earlier cited. Further investigation of this point, however, is being undertaken.

Group IV corresponds with the pair of small chromosomes in that it contains so few factors. For this reason, the author, in his account of the inheritance of bent wing, in 1914, said:

It also seems probable that when other mutations are discovered in the fourth group, the genes in which they occur will be found to be linked strongly to the gene for bent wings, since the fourth chromosome is probably the small one, and so any genes in it must lie near together.

One other mutant factor, "eyeless," has since been found, by Miss Hoge, to lie in this group. But although Miss Hoge has made numerous attempts (3) to combine eye-

less and bent, no recombinations between them have so far been obtainable. Group IV, therefore, forms a marked contrast to all the other groups as regards the frequency of separation within it, and this result is the more striking, not only because it shows that there is a group of factors corresponding in separation frequency to the pair of short chromosomes, but also because it happens that this group is the same one as that which had previously been identified with the pair of short chromosomes by reason of the fewness of the mutant factors discovered in it.

It is therefore evident, not only that the relative sizes of the chromosomes are in a general way like the separation frequencies of the groups, but also that where there is evidence from another source indicating in which chromosome a certain group lies, this is the very one to which the group corresponds by its total frequency of separation. It has been shown that this is true in the case of the fourth group. In the case of the first group, the sex-linked inheritance of the latter connects it with the X-chromosome, and since this is the moderately long chromosome, it is just this one with which group I would be identified by its frequency of separation. The other two groups, both of which are long—one certainly very long, and the other probably so—are thus left to correspond with the remaining chromosomes, both of which are very long and indistinguishable in appearance.

In the remainder of this article, therefore, the word ''chromosome'' will be used instead of ''group'' and ''crossing-over'' instead of ''separation of linked factors.''